[55] 54. (Renumbered) A method for construction of a ribozyme vector, said method comprising:

contacting a single-stranded oligonucleotide with a linearized delivery vector wherein said single-stranded oligonucleotide or a strand that is complementary to said single-stranded oligonucleotide encodes a transcription product that is a ribozyme, wherein said single-stranded oligonucleotide is complementary to one end of said delivery vector and base pairs with said delivery vector, and wherein said strand that is complementary to said single-stranded oligonucleotide is formed with a DNA polymerase , wherein said ribozyme vector encodes a ribozyme which cleaves a mRNA transcribed from a target nucleic acid, and wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

[56] 55. (Renumbered and Amended) The method according to Claim [55] 54, wherein said DNA polymerase is Klenow.

[57] 56. (Renumbered and Amended) The method according Claim [43] 42 or Claim [55] 54, wherein said ribozyme vector further comprises regulatory elements for expression.

REMARKS

The Amendments

Claim 41 has been cancelled and Claims 42-57 renumbered in consecutive order, starting with 41.

Claim 1 is amended to recite "obtaining host cells that identify the function of" and "expressing one or more members of an oligonucleotide family in a high-throughput format as individual transcription products in a multiplicity of recombinant non-bacterial host cells, wherein the coding sequence for said individual transcription products is contained in an expression vector lacking bacterial or bacteriophage cloning sequences and codes for an antisense nucleic acid which when expressed as RNA binds to a mRNA sequence transcribed from a target RNA sequence that comprises a nucleotide sequence of said sample nucleic acid, wherein expression of one or more of said individual transcription products prevents production of a product of said mRNA; and isolating host cells that have an altered phenotype". Support for the amendment is found, for example, at page 4, line 25 to page 5, line 5.

Claim 4 is amended to recite "host cells". Support for the amendment is found, for example, at page 4, lines 25-28.

Claim 7 is amended to delete "essential" and to recite "expressed sequence tag" in order to conform EST to its standard terminology as suggested by the Examiner. Use of the term "essential" instead of "expressed" was due to a clerical error.

Claim 8 is amended to recite "host cells obtained according to the method of Claim 1 as indicative of the function of a product of said sample nucleic acid" and "a product of said sample nucleotide sequence". Support for the amendment is found, for example, at page 4, line 25 to page 5, line 5.

Claim 14 is amended to recite "said altered function is monitored directly". Support for the amendment is found, for example, at page 15, lines 30-31.

Claim 15 is amended to recite "An expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a mammalian target nucleic acid sequence so that expression of a product of said mammalian target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA; wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences." Support for the amendment is found, for example, at page 9, line 14 to page 11, line 4.

Claims 16-23 are amended to recite "expression vector". Support for the amendment is found, for example, at page 10, line 28 to page 11, line 4.

Claim 24 is amended to recite "A triple-stranded oligonucleotide, wherein said triple-stranded oligonucleotide is by the method of". Support for the amendment is found, for example, at page 8, lines 5-28.

Claims 26-27 are amended to recite "delivery vector". Support for the amendment is found, for example, at page 9, line 28.

Claims 31, 35, and 37, and new Claim 44 are amended to recite "wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA". Support for the amendments is found, for example, at page 6, lines 4-7.

Claim 32 is amended to recite "Host cells obtained according to the method of Claim 1, wherein said host cells are mammalian host cells." Support for the amendments is found, for example, at page 4, line 25 to page 5, line 5.

Claims 33 and 36 is amended to recite "comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA, wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences". Support for the amendments is found, for example, at page 7, line 18 to page 9, line 29.

Claim 34 is amended to recite "adeno-associated expression vector". Support for the amendments is found, for example, at page 12, lines 11-15.

Claim 40 is amended to recite "wherein said plasmid expression vector is expressed in said host cell(s) without an intervening bacterial cloning step". Support for the amendment is found, for example, at page 6, lines 9-12.

New Claim 44 is amended to recite "wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences". Support for the amendment is found, for example, at page 6, lines 4-7.

New Claim 54 is amended to recite wherein said ribozyme vector encodes a ribozyme which cleaves a mRNA transcribed from a target nucleic acid, and wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA". Support for the amendments is found, for example, at page 6, lines 4-7.

New Claims 45-53, 55, and 56 are amended to refer to the correct antecedent claims.

Response

The claims have been renumbered as follows:

Old Claim No.	New Claim No.	Old Claim No.	New Claim No.
1	1	31	31
2	2	32	32

3	3	33	33
4	4	34	34
5	5	35	35
6	6	36	36
7	7	37	37
8	8	38	38
9	9	39	39
10	10	40	40
11	11	41	--
12	12	42	41
13	13	43	42
14	14	44	43
15	15	45	44
16	16	46	45
17	17	47	46
18	18	48	47
19	19	49	48
20	20	50	49
21	21	51	50
22	22	52	51
23	23	53	52
24	24	54	53
25	25	55	54
26	26	56	55
27	27	57	56
28	28		
29	29		
30	30		

1. <u>Reasoned Statement Under Rule 66.2(a)(ii)</u>

<u>Claims 1 and 4-7 (as amended) are novel</u>

Applicants respectfully traverse the Examiner's assertion that Claims 1 and 4-7 (as amended) are not novel, because the cited references do not disclose a method of obtaining cells that can be used to identify the function of a product of a sample nucleic acid. Sambrook et al. disclose only the expression of proteins in eukaryotic cells (p. 16.3), the introduction of recombinant vectors into mammalian cells (p. 16.30), strategies for studying gene regulation in mammalian cells (p. 16.56), and cloning by expression in mammalian cells (p. 16.68). These procedures disclosed by Sambrook et al. all use an amplification step of DNA in bacteria prior to introduction into eukaryotic cells. This is in contrast to the claimed method which does not use a bacterial cloning step: Sambrook et al. disclose using mammalian expression vectors that contain "Prokaryotic Plasmid Sequences That Facilitate the Construction, Propagation, and

Amplification of Recombinant Vector Sequences in Bacteria" (p. 16.5) and that "resulting vector[s are] then amplified in bacteria before being transfected into cultured mammalian cells." (p. 16.17). Claim 1 as amended specifically recites that the expression vector lacks bacterial cloning sequences. Therefore, Claim 1 and 4-7 are novel as required by Article 33(2) PCT.

<u>Claim 2 (as amended) is novel</u>

DE 4424762C (D1) discloses a method of cleaving the mRNA of human growth hormone in CHO cells using a vector encoding a ribozyme which cleaves the mRNA of hGH (col. 5, line 65 to col. 6, line 8).

WO 9201786A (D2) discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the (-) RNA of PPV (p. 40, lines 12-22) as well as ribozyme directed against the mRNA of the ice nucleation gene (in a Z), the hrpS gene of *Pseudomonas syringae*, the white gene of D. *melanogaster*, the transcriptional regulator of GCN4 of yeast and the (+) and the (-) RNA of Sendai virus (p. 47). The purpose of making these DNA was to inhibit production of a known product of a non-mammalian gene.

WO 9609392A (D3) discloses a method of cleaving apo(a) mRNA *in vitro* using an engineered ribozyme motifs (p. 14, lines 10-13).

None of these references discloses a method for obtaining host cells that identify the function of a product coded for by a sample nucleic acid. In fact, D2-3 do not even disclose recombinant non-bacterial cell cultures containing an expression vector comprising at least one member of a oligonucleotide family. D1-3 also do not disclose a method which lacks a bacterial cloning step of the one or more members of the oligonucleotide family. Therefore, Claim 2 is not anticipated by D1-3 and is novel as required by Article 33(2) PCT.

<u>Claim 3 is novel</u>

WO 9420618A (D4) discloses a method of obtaining genetic suppressor elements ("GSE") that confer resistance to etoposide by transfecting HeLa cells with a random fragment expressing library (p. 16, line 14 to p. 17, line 2). D4 discloses constructing the library of GSE through cloning into *E. coli* to obtain a population of plasmids (p. 20, lines 7-9). Therefore, D4 does not anticipate Claim 3 for two reasons: (1) D4 does not disclose a method for obtaining host cells that identify the function of a product coded for by a sample nucleic acid, and (2) D4 does

not disclose a method which lacks a bacterial cloning step of the one or more members of the oligonucleotide family. Therefore, Claim 3 is not anticipated by D4 and is novel as required by Article 33(2) PCT.

Claims 8-14 involve an inventive step

The inventive step of Claims 8-14 is the use of a family of nucleic acids (exemplified by ribozymes in the instant application) to cause host cells, expressing the sample nucleotide sequence, to exhibit at least one phenotypic change so that these phenotypic changes can be analyzed and a function(s) assigned to a sample nucleotide sequence.

D1 discloses a method of cleaving the mRNA of human growth hormone in CHO cells using a vector encoding a ribozyme which cleaves the mRNA of hGH (col. 5, line 65 to col. 6, line 8).

D2 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the (-) RNA of PPV (p. 40, lines 12-22) as well as ribozyme directed against the mRNA of the ice nucleation gene (in a Z), the hrpS gene of *Pseudomonas syringae*, the white gene of D. *melanogaster*, the transcriptional regulator of GCN4 of yeast and the (+) and the (-) RNA of Sendai virus (p. 47).

D3 discloses a method of cleaving apo(a) mRNA *in vitro* using an engineered ribozyme motifs (p. 14, lines 10-13).

D4 discloses a method of obtaining GSE that confers resistance to etoposide by transfecting HeLa cells with a random fragment expressing library (p. 16, line 14 to p. 17, line 2).

D1-4 merely disclose methods of inhibiting the products of known target nucleic acids using ribozymes or antisense RNA. None of these references disclose a method of assigning a function to a product coded for by a target nucleic acid. Claims 8-14 are directed to a method whereby a function(s) is assigned to a target nucleic acid.

The Examiner asserts that the principle of inhibiting expression of a certain gene in order to identify the function of said gene is applied in many fields of molecular biology and methods derived from this principle are known as site-directed mutagenesis, gene ablation, and knock-out transgenesis. The methods cited by the Examiner all involve the removal or alteration of a known target gene(s) itself. However, the claimed method does not involve removing or altering

of a target gene, but instead uses a family of nucleic acids (that code for e.g. a ribozyme or an antisense RNA) to inhibit expression from a target nucleic acid by cleaving the mRNA in order to determine the function of the sequence from which the RNA is transcribed. The methods disclosed by the references are entirely different from the claimed method. Therefore, Claims 8-14 are not rendered obvious by D1-4, and involve an inventive step as required by Article 33(3) PCT.

Claims 15-22, and 26-40, and renumbered Claims 41, 42, and 56 (as amended) are novel

D1 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the mRNA of hGH (col. 3, line 66 to col. 4, line 32).

D2 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the (-) RNA of PPV (p. 40, lines 12-22) as well as ribozyme directed against the mRNA of the ice nucleation gene (in a Z), the hrpS gene of *Pseudomonas syringae*, the white gene of D. *melanogaster*, the transcriptional regulator of GCN4 of yeast and the (+) and the (-) RNA of Sendai virus (p. 47). The purpose of making these DNA was to inhibit production of a known product of a non-mammalian gene.

D3 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves apo(a) mRNA (p. 13, lines 33-35). However, D3 does not disclose that the product of apo(a) mRNA is associated with at least one phenotypic property of a host cell comprising apo(a) mRNA. In contrast, the claimed DNA is to determine the function of an unknown nucleic acid sequence.

D4 discloses double stranded DNA that encodes GSE that confers resistance on HeLa cells to etoposide (pp. 15-16, Example 1). However, D4 does not disclose that the GSE binds a mRNA transcribed from a target nucleic acid so that expression of a product of the target nucleic acid is inhibited, or that the product of the such a mRNA is associated with at least one phenotypic property of a host cell comprising the mRNA.

D1-4 merely disclose the cleavage of mRNA of human growth hormone (D1), transcripts of non-mammalian genes (D2), and apo(a) mRNA (D3), and the conferral of resistance to etoposide on HeLa (D4). Unlike Claim 15, D1-4 do not disclose an expression vector that comprises a sense strand that codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a

product of said target nucleic acid is inhibited, wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence and wherein said expression vector lacks bacterial or bacteriophage cloning sequences. Claim 8 is novel in that the product of the target nucleic acid is associated with at least one phenotypic property of a host cell comprising the mRNA sequence and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

Claims 16-22, 26-27 are directed to an expression vector according to Claim 15. Therefore since Claim 15 is novel, and Claims 16-20 depend from Claim 15, then Claims 16-20 are also novel.

Claims 28-29 are directed to a retrovirus expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said retrovirus expression vector lacks bacterial or bacteriophage cloning sequences. Since the expression vectors of D1-4 contain either bacterial or bacteriophage cloning sequences, D1-4 do not anticipate the claimed retrovirus expression vector, therefore Claims 28-29 are novel.

Claim 30 is directed to a retrovirus packaging cell line comprising the retrovirus expression vector according to Claim 28. Since D1-4 do not anticipate the retrovirus expression vector according to Claim 28, D1-4 do not anticipate a retrovirus packaging cell line comprising such a retrovirus expression vector. Therefore Claim 30 is novel.

Claim 31 is directed to retrovirus particle comprising a genome encoding expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA. For the reasons stated earlier, D1-4 do not disclose a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA. Therefore Claim 31 is novel.

Claim 32 is directed to host cells obtained according to Claim 1, wherein said host cells are mammalian cells. For the reasons stated earlier, since D1-4 do not anticipate Claim 1, therefore D1-4 do not anticipate Claim 32. Therefore Claim 32 is novel.

Claim 33 is directed to an adeno-associated virus expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA, wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences. D1-4 do not anticipate the claimed adeno-associated virus expression virus vector. Therefore Claim 33 is novel.

Claim 34 is directed to an adeno-associated packaging cell line comprising an adeno-associated virus expression vector according to Claim 33. Since D1-4 do not anticipate the adeno-associated virus expression vector according to Claim 33, D1-4 do not anticipate an adeno-associated packaging cell line comprising such a adeno-associated virus expression vector. Therefore Claim 34 is novel.

Claim 35 is directed to an adeno-associated virus comprising a genome encoding expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, wherein said RNA is associated with at least one phenotypic property of a host cell comprising said mRNA. Since D1-4 do not disclose the claimed adeno-associated virus. Therefore Claim 35 is novel.

Claim 36 is directed to a plasmid expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences. D1-4 do not anticipate the claimed plasmid expression vector comprising a double-stranded DNA. Therefore Claim 36 is novel.

Claims 37-39 are directed to a method for introducing into a host cell(s) a plasmid expression vector by contacting a cell culture comprising one or more host cell(s) with a co-precipitate of calcium phosphate and a double-stranded DNA encoding a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

D1 discloses a method of transfecting CHO cells with pCMVhGH, wherein pCMVhGH encodes a ribozyme that cleaves the mRNA of hGH (col. 5, line 68 to col. 6, line 1). D1 discloses transfecting CHO cells with a vector, therefore D1 does not disclose a method in which a cell culture is contacted with a co-precipitate of calcium phosphate and a double-stranded DNA.

D2 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the (-) RNA of PPV (p. 40, lines 12-22) as well as ribozymes directed against the mRNA of the ice nucleation gene (inaZ), the hrpS gene of *Pseudomonas syringae*, the white gene of D. *melanogaster*, the transcriptional regulator of GCN4 of yeast and the (+) and the (-) RNA of Sendai virus (p. 47). The purpose of making these DNA was to inhibit production of a known product of a non-mammalian gene. D2 does not disclose the claimed method wherein a cell culture is contacted with a co-precipitate of calcium phosphate and a double-stranded DNA.

D3 discloses a method of cleaving apo(a) mRNA *in vitro* using engineered ribozyme motifs (p. 14, lines 10-13). D3 discloses the cleaving of apo(a) mRNA by a ribozyme *in vitro*, therefore D3 does not disclose a method where a vector is introduced into a host cell. D3 also does not disclose a method in which a cell culture is contacted with a co-precipitate of calcium phosphate and a double-stranded DNA.

D4 discloses a method of obtaining GSE that confers resistance to etoposide by transfecting HeLa cells with a random fragment expressing library (p. 16, line 14 to p. 17, line 2). D4 does not disclose a method where a vector is introduced into a host cell by contacting the cell culture with a co-precipitate of calcium phosphate and a double-stranded DNA. D4 discloses a method which uses GSE which when expressed does not cleave RNA, therefore D4 does not disclose a method which uses a catalytic domain which when expressed as RNA cleaves a mRNA sequence.

Therefore D1-4 do not anticipate Claims 37-39.

Claim 40 (as amended) and new Claims 41-42, and 56 are directed to a method for expressing in a host cell a plasmid expression vector by introducing into said host cell said plasmid expression vector comprising a double-stranded DNA encoding a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, wherein said mRNA sequence is associated with at least one phenotypic property of a host cell comprising said mRNA.

D1 does not disclose a method of introducing into a host cell a double-stranded DNA encoding a catalytic domain that cleaves a mRNA wherein the mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA. D2-3 do not disclose a method where a vector is introduced into a host cell. D4 does not disclose a method which uses a catalytic domain which when expressed as RNA cleaves a mRNA sequence.

Claim 40 is amended to incorporate the limitation of old Claim 41, which the Examiner has stated is novel and inventive. Therefore Claim 40 (as amended), and new Claims 41-42 and 56, which depend from Claim 40, are not anticipated by D1-4 and are novel and inventive.

Therefore, Claims 15-22, 26-40, and new Claims 42, 43, and 57 are not anticipated by D1-4 and are novel as required by Article 33(2) PCT.

Claims 23-25 involve an inventive step

Claim 23 is directed to an expression vector comprising double-stranded DNA wherein said double-stranded DNA is formed is formed by contacting a triple-stranded oligonucleotide with an expression vector. Claims 24-25 are directed to the triple-stranded oligonucleotide. The Examiner asserts that the combination of any of the teachings of D1-4 and standard triplex technology will result in the claimed subject-matter. However, none of the references disclose or suggest the use of a triple-stranded oligonucleotide to form a double-stranded DNA which encodes an antisense strand which when expressed as RNA binds a mRNA sequence. None of the references disclose inhibiting a mRNA sequence which encodes a product which is associated with at least one phenotypic property of a host cell comprising the mRNA sequence. Therefore, Claims 23-25 involve an inventive step as required by Article 33(3) PCT.

New Claim 43 is novel

New Claim 43 is directed to the method according to Claim 40, wherein the plasmid expression vector is contacted with gyrase. Claim 40 has been amended to incorporate the limitation of old Claim 41, which the Examiner has stated is novel and inventive. Therefore new Claim 43, which depends from new Claim 40, is not anticipated by D1-4 and is novel and inventive as required by Article 33 PCT.

New Claims 44-47 are novel

New Claims 44-47 are directed to a method for construction of a ribozyme vector by inserting a double-stranded DNA into a linearized delivery vector, wherein the double-stranded DNA comprises a sense strand which encodes a catalytic domain which when expressed as RNA cleaves a mRNA, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences.

D1 discloses a method of cloning a fragment encoding a ribozyme that cleaves mRNA of hGH into the pGEMT plasmid (col. 5, lines 19-20). D1 does not disclose the ribozyme cleaves a mRNA sequence transcribed from a target nucleic acid wherein the mRNA sequence is associated with at least one phenotypic property of a host cell comprising the mRNA of hGH.

D2 discloses a double stranded DNA that encodes a ribozyme that binds and cleaves the (-) RNA of PPV (p. 40, lines 12-22) as well as ribozymes directed against the mRNA of the ice nucleation gene (in a Z) or the hrpS gene of *Pseudomonas syringae*, the white gene of D. *melanogaster*, the transcriptional regulator of GCN4 of yeast and the (+) and the (-) RNA of Sendai virus (p. 47). The purpose of making these DNA was to inhibit production of a known product of a non-mammalian gene.

D3 discloses inserting a transcription unit expressing a ribozyme that cleaves apo(a) RNA into a plasmid DNA vector (p. 13, lines 33-35). D3 does not disclose that the product of apo(a) mRNA is associated with at least one phenotypic property of a host cell comprising apo(a) mRNA.

D1-3 merely disclose methods of cloning a fragment encoding a ribozyme that cleaves the mRNA of human growth hormone (D1), mRNA of non-mammalian genes (D2), and apo(a)

mRNA (D3). Unlike new Claims 44-47, D1-3 do not disclose a method wherein the ribozyme cleaves a mRNA sequence transcribed from a target nucleic acid wherein the mRNA sequence is associated with at least one phenotypic property of a host cell comprising the mRNA, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences.

Therefore, new Claims 44-47 are not anticipated by D1-3 and are novel as required by Article 33(2) PCT.

New Claims 48-53 involve an inventive step

New Claims 48-53 depend from new Claim 44. For the reasons stated earlier, new Claim 44 is not anticipated by D1-3. New Claims 48-53 are directed a method of construction of a ribozyme vector by inserting a double-stranded DNA into a linearized delivery vector, where the double-stranded DNA is formed by annealing three oligonucleotides. D1-3 merely disclose methods of cloning a fragment encoding a ribozyme that cleaves the mRNA of human growth hormone (D1), transcripts of non-mammalian genes (D2), and apo(a) mRNA (D3). D1-3 do not disclose or suggest a method wherein the ribozyme cleaves a mRNA sequence transcribed from a target nucleic acid wherein the mRNA sequence is associated with at least one phenotypic property of a host cell comprising the mRNA wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences. Therefore, new Claims 48-53 involve an inventive step as required by Article 33(3) PCT.

New Claims 54, 55, and 56 involve an inventive Step

New Claims 54, 55, and 56 are directed to a method for construction of a ribozyme vector by contacting a single-stranded oligonucleotide with a linearized delivery vector wherein said single-stranded oligonucleotide, or its complementary strand, encodes a ribozyme, wherein said single-stranded oligonucleotide is complementary to one end of said delivery vector and base pairs with said delivery vector and wherein said strand that is complementary to said single-stranded oligonucleotide is formed with a DNA polymerase, wherein said ribozyme vector encodes a ribozyme which cleaves a mRNA transcribed from a target nucleic acid, and wherein

said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

As the Examiner has stated, D1-3 disclose methods for the construction of vectors comprising ribozymes wherein the nucleic acid comprising the ribozyme function is ligated to the vector as double-stranded molecule. D1-3 do not disclose or suggest contacting single-stranded oligonucleotides with a linearized delivery vector in order to construct a ribozyme vector. The Examiner has neither provided any evidence that the method of using a single-stranded oligonucleotide is "customary practice in the field of molecular cloning" nor the motivation for one skilled in the art to combine such a technique with the disclosures of D1-3.

In addition, D1-3 merely disclose methods of cloning a fragment encoding a ribozyme that cleaves the mRNA of human growth hormone (D1), transcripts of non-mammalian genes (D2), and apo(a) mRNA (D3). D1-3 do not disclose or suggest a method wherein the ribozyme cleaves a mRNA transcribed from a target nucleic acid wherein the mRNA is associated with at least one phenotypic property of a host cell comprising the mRNA.

Therefore, new Claims 54, 55, and 56 involve an inventive step as required by Article 33(3) PCT.

3. <u>Certain Observations</u>

Claims 1, 15, 37, and 40 have been amended to include the essential features of the invention. Claim 8 contain the essential feature of the invention which is the use of one or more members of a family of nucleic acids which bind to a transcription product whereby the transcription product is inhibited and a host cell, expressing the transcription product, exhibits at least one phenotypic property.

Claim 14 has been amended so that the definition of the subject-matter is not vague and unclear.

Claim 7 has been amended so that term "essential" is deleted and the term "expressed" is substituted in order to correct a clerical error. The amendment of the claim renders the subject-matter of the claim clear.

The subject-matter for which protection is sought is defined by the claims. The recitation "herein incorporated by reference" does not imply the subject-matter defined by the claims is

anything other than what the claims recite. The recitation does not imply that the extent of protection may be expanded in some vague and not precisely defined way.

CONCLUSION

In view of the above amendment and remarks, it is submitted that this application has overcome all the rejections and objections cited in the Written Opinion and has novelty, inventive step and industrial applicability. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

A copy of the claims as replacement pages 29 to 36 is attached hereto.

Respectfully submitted,

Dated: March 8, 2000

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1. A method for obtaining host cells that identify the function of a product of a sample nucleic acid, said method comprising the steps of:

expressing one or more members of an oligonucleotide family in a high-throughput format as individual transcription products in a multiplicity of recombinant non-bacterial host cells, wherein the coding sequence for said individual transcription products is contained in an expression vector lacking bacterial or bacteriophage cloning sequences and codes for an antisense nucleic acid which when expressed as RNA binds to a mRNA sequence transcribed from a target RNA sequence that comprises a nucleotide sequence of said sample nucleic acid, wherein expression of one or more of said individual transcription products prevents production of a product of said mRNA; and

isolating host cells that have an altered phenotype.

2. The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is a ribozyme.

3. The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is an antisense nucleic acid.

4. The method according to Claim 1, wherein said recombinant non-bacterial host cells comprise mammalian cells.

5. The method according to Claim 1, wherein said expression vector is a plasmid or a virus.

6. The method according to Claim 5, wherein said virus is a retrovirus, or an adeno-associated virus.

7. The method according to Claim 1, wherein said sample nucleic acid is a genomic DNA, a cDNA, an expressed sequence tag (EST), or an RNA.

8. A method of assigning a function to a product coded for by a sample nucleotide sequence, said method comprising:

analyzing phenotypic changes in host cells obtained according to the method of Claim 1 as indicative of the function of a product of said sample nucleic acid; and

obtaining a nucleotide sequence of said target nucleic acid, whereby a function is assigned to a product of said sample nucleotide sequence.

9. The method according to Claim 8, wherein said function is a physiological function.

10. The method according to Claim 8, wherein said function is enzyme activity.

11. The method according to Claim 8, wherein said function is protein synthesis.

12. The method according to Claim 8, wherein said function is expression of a biological factor.

13. The method according to Claim 8, wherein said function is a regulatory effector function.

14. The method according to Claim 8, wherein said altered function is monitored directly.

15. An expression vector comprising:

a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a mammalian target nucleic acid sequence so that expression of a product of said mammalian target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA; wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

16. The expression vector according to Claim 15, wherein said RNA comprises a

a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA.

17. The expression vector according to Claim 16, wherein said RNA is a ribozyme.

18. The expression vector according to Claim 15, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

19. The expression vector according to Claim 18, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

20. The expression vector according to Claim 19, wherein said non blunt-ended restriction enzyme site is complementary to an end of said expression vector.

21. An expression vector according to Claim 15, wherein said expression vector is formed by contacting a double-stranded oligonucleotide with an expression vector.

22. An expression vector according to Claim 15, wherein said expression vector is formed by contacting a single-stranded oligonucleotide with said expression vector.

23. An expression vector according to Claim 15, wherein said double-stranded DNA is formed by contacting a triple-stranded oligonucleotide with an expression vector.

24. A triple-stranded oligonucleotide, wherein said triple-stranded oligonucleotide is by the method of contacting a first oligonucleotide, a second oligonucleotide and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

25. The triple-stranded oligonucleotide according to Claim 24, wherein said excess nucleotides are complementary to and base pair with the ends of said expression vector.

26. The delivery vector according to Claim 22 or Claim 23, wherein said expression vector is filled in with Klenow.

27. The delivery vector according to Claim 21, Claim 22, or Claim 23, wherein said expression vector further comprises regulatory elements for expression.

28. A retrovirus expression vector comprising:

a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said retrovirus expression vector lacks bacterial or bacteriophage cloning sequences.

29. The retrovirus expression vector according to Claim 28, wherein said vector comprises supercoiled DNA.

30. A retrovirus packaging cell line comprising:

a retrovirus expression vector according to Claim 28.

31. A retrovirus particle comprising:

a genome encoding expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

32. Host cells obtained according to the method of Claim 1, wherein said host cells are mammalian host cells.

33. An adeno-associated virus expression vector comprising:

a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

34. An adeno-associated virus packaging cell line comprising:

an adeno-associated virus expression vector according to Claim 33 and an adeno-associated virus helper plasmid.

35. An adeno-associated virus comprising:

a genome encoding the expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

36. A plasmid expression vector comprising:

a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA , wherein said product is associated with at least one phenotypic property of a host cell comprising said mRNA sequence; and wherein said expression vector lacks bacterial or bacteriophage cloning sequences.

37. A method for the introduction into a host cell(s) of a plasmid expression vector, said method comprising:

contacting a cell culture comprising one or more host cell(s) with a co-precipitate of calcium phosphate and a double-stranded DNA comprising a sense strand and an antisense

RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, wherein a means for determining directionality of expression is included in said double-stranded DNA, and wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, whereby said plasmid expression vector is introduced into said host cells.

38. The method according to Claim 37, wherein said host cell(s) comprise mammalian cell(s).

39. The method according to Claim 37, wherein said plasmid expression vector is supercoiled DNA.

40. A method for expressing in a host cell a plasmid expression vector, said method comprising:

introducing into said host cell said plasmid expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA, wherein a means for determining directionality of expression is included in said double-stranded DNA, and wherein said plasmid expression vector lacks bacterial or bacteriophage cloning sequences, whereby said plasmid expression vector is expressed in said host cells.

41. The method according to Claim 40, wherein said plasmid expression vector is a retrovirus expression vector.

42. The method according to Claim 40, wherein said plasmid expression vector is an adeno-associated virus expression vector.

43. The method according to Claim 40, wherein said plasmid expression vector is contacted with gyrase.

44. A method for construction of a ribozyme vector, said method comprising:

inserting a double-stranded DNA into a linearized delivery vector, wherein said double-stranded DNA comprises a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA , wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA, and wherein said ribozyme vector lacks bacterial or bacteriophage cloning sequences.

45. The method according to Claim 44, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

46. The method according to Claim 45, wherein a means for determining directionality of expression is included in said double-stranded DNA.

47. The method according to Claim 46, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

48. The method according to Claim 44, wherein said double-stranded DNA is formed by annealing a first oligonucleotide, a second oligonucleotide, and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

49. The method according to Claim 48, wherein said excess nucleotides are complementary to and base pair with the ends of said linearized delivery vector.

50. The method according to Claim 48, wherein said ribozyme vector is treated with a DNA polymerase

51. The method according to Claim 50, wherein said DNA polymerase is a cellular DNA polymerase.

52. The method according to Claim 50, wherein said DNA polymerase is a Taq DNA polymerase.

53. The method according to Claim 50, wherein said DNA polymerase is Klenow.

54. A method for construction of a ribozyme vector, said method comprising:

contacting a single-stranded oligonucleotide with a linearized delivery vector wherein said single-stranded oligonucleotide or a strand that is complementary to said single-stranded oligonucleotide encodes a transcription product that is a ribozyme, wherein said single-stranded oligonucleotide is complementary to one end of said delivery vector and base pairs with said delivery vector, and wherein said strand that is complementary to said single-stranded oligonucleotide is formed with a DNA polymerase, wherein said ribozyme vector encodes a ribozyme which cleaves a mRNA transcribed from a target nucleic acid, and wherein said mRNA is associated with at least one phenotypic property of a host cell comprising said mRNA.

55. The method according to Claim 54, wherein said DNA polymerase is Klenow.

56. The method according Claim 42 or Claim 54, wherein said ribozyme vector further comprises regulatory elements for expression.